ESTCP
Cost and Performance Report

(RC-201205)

Monitoring Species of Concern Using Noninvasive Genetic Sampling and Capture-Recapture Methods

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4. ABSTRACT
hrough this project we demonstrated the effectiveness of an approach utilizing noninvasive genetic sampling combined ith capture-recapture methods (NGS-CR) to evaluate the status of kit fox (*Vulpes macrotis*) populations on Dugway roving Ground (DPG) and Sonoran pronghorn (*Antilocapra americana sonoriensis*) on the Barry M. Goldwater Range BMGR). We compared the reliability of demographic parameters and the cost-efficiency of NGS-CR approaches with lternative approaches. We developed a spatio-temporal sampling design for acquiring noninvasive genetic data (via fecal cats) from individuals, genotyped samples for individual identification, analyzed genotypes with capture-recapture ethods to obtain estimates of key population parameters, and developed a protocol for long-term monitoring in the future. We also quantified expenditures to examine cost efficiency of the approach. For both species, we concluded the NGS-CR ethod provided reliable estimates, improved monitoring (i.e., increased the number of demographic parameters stimates), and improved monitoring efficiency. Survey results indicated managers and technicians NGS-CR methods ere easier to implement than traditional methods (e.g., radio telemetry). Managers at these installations have expressed terest in continuing using these NGS-CR methods.

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ACRONYMS AND ABBREVIATIONS

AICc	Akaike's Information Criterion with small sample size correction
AZGFD	Arizona Game and Fish Department
BMGR	Barry M. Goldwater Range
c	Probability of Recapture
CAPWIRE	Capture with Replacement
CI	Confidence Interval
CPNWR	Cabeza Prieta National Wildlife Refuge
D	Estimated Density
DoD	Department of Defense
DNA	Deoxyribonucleic Acid
DPG	Dugway Proving Ground
ε	Probability of local extinction
ESA	Endangered Species Act
ESTCP	Environmental Security Technology Certification Program
γ	Probability of colonization
GIS	Geographic Information Systems
g0	Detection Function Intercept
ID	Identification
INRMPs	Integrated Natural Resource Management Plans
LAFB	Luke Air Force Base
MNKA	Minimum Number Known Alive
N	Abundance
N_e	Effective Population Size
NGS	Noninvasive Genetic Sampling
NGS-CR	Noninvasive Genetic Sampling – Capture Recapture
NGS-OM	Noninvasive Genetic Sampling – Occupancy Modeling
NWR	National Wildlife Refuge
p	Probability of Capture
PCR	Polymerase chain reaction
ψ	Probability of occurrence (occupancy)
σ	Detection Function Scale Parameter

RMSE	Relative Mean Squared Error
S	Probability of Survival
S_F	Female Probability of Survival
S_M	Male Probability of Survival
SECR	Spatially Explicit Capture-Recapture
σ	Detection Function Scale Parameter
SERDP	Strategic Environmental Research and Development Program
USFWS	United States Fish and Wildlife Service
USU	Utah State University

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EXECUTIVE SUMMARY

OBJECTIVES OF THE DEMONSTRATION

Our primary objective was to demonstrate how noninvasive genetic sampling (NGS) could be combined with capture-recapture (NGS-CR) modeling to evaluate the status of species of conservation concern. A secondary objective was to demonstrate the combination of NGS with occupancy modeling (NGS-OM) to estimate the proportion of area occupied (i.e., occupancy) and patterns of local extinction and colonization. We evaluated the efficacy of NGS as a viable, long-term monitoring approach for two species on Department of Defense (DoD) installations: the kit fox (*Vulpes macrotis*) (Dugway Proving Ground [DPG]), a species of concern for western installations, and Sonoran pronghorn (*Antilocapra americana sonoriensis*) (Barry M. Goldwater Range [BMGR]), an endangered subspecies of North American pronghorn that occurs in southern Arizona. For both species, we developed a spatio-temporal sampling design for acquiring noninvasive genetic data (via fecal scats), genotyped samples for individual ID, analyzed genotypes with capture-recapture methods to obtain estimates of population parameters, and developed a protocol for long-term monitoring in the future. We also quantified expenditures to examine cost efficiency of the approach. Additionally, we evaluated NGS-OM only for kit foxes, and its sympatric intraguild predator, the coyote (*Canis latrans*). We monitored kit foxes and coyotes simultaneously, used genetic analyses (via scats) to confirm species, and employed dynamic occupancy modeling to obtain estimates of detection, proportion of area occupied, and local colonization and extinction for each species, and to evaluate the influence of coyotes and landscape features on kit fox space-use.

Our performance objectives were to (1) improve monitoring protocols for kit foxes and Sonoran pronghorn based on NGS-CR, (2) obtain reliable estimates of demographic parameters from NGS-CR for each species, (3) improve efficiency of current monitoring programs, (4) evaluate ease of use, (5) obtain estimates of occupancy and dynamic parameters (i.e., local colonization and extinction) from NGS-OM for kit foxes, and (6) facilitate transference of monitoring programs for kit foxes and Sonoran pronghorn based on NGS-CR.

TECHNOLOGY DESCRIPTION

Capture-recapture modeling has been commonly used for estimating wildlife population parameters. The theory is based on modeling capture and recapture probabilities of populations or individuals as a function of population size, survival, reproduction, and movements among populations. The process involves capturing individuals and marking them, such that on subsequent capture occasions, marked individuals can be identified. Using the observed capture histories, demographic parameters, such as abundance, survival, reproduction, immigration, or emigration, can be estimated. We employed Pollock's robust design (Pollock et al. 1990, Kendall et al. 1997) capture-recapture models. Additionally, we employed single session 'capture with replacement' (CAPWIRE) models. CAPWIRE exploits repeat detections of individuals within a single sampling occasion to generate abundance estimates (Miller et al. 2005). For kit fox, we also employed spatially explicit capture-recapture (SECR) models.

Occupancy modeling utilizes information from repeat surveys to account for imperfect detection and produces unbiased estimates of occupancy (MacKenzie et al. 2002, 2003, 2006). Unlike NGS-CR, the unit of analysis in occupancy studies is the survey site (or patch) not the individual.

Consequently, patterns of occurrence can be modeled as a function of patch characteristics, such as habitat or landscape features. Replication is required to estimate probability of detection for occupancy models and may be accomplished through temporal or spatial replicates within a site. One benefit of NGS-OM is that it requires only species' ID of noninvasively collected genetic samples, and subsequently may offer a more affordable monitoring strategy if estimates of abundance and survival are not required.

DEMONSTRATION RESULTS

All performance objectives were met. Across sessions, 109 kit foxes were identified. We captured 36–50 kit foxes each session. We captured more males (60%) than females. Male kit fox probability of survival (S_M) was slightly lower than female probability of survival (S_F) across intervals and overall. Model-averaged kit fox survival was high in the period between winter 2013 and summer 2013 (S_M = 0.82, 95% Confidence Interval [CI] = 0.26–0.98; S_F = 0.87, 95% CI = 0.28–0.99), high between summer 2013 and winter 2014 (S_M = 0.81, 95% CI = 0.19–0.98; S_F = 0.87, 95% CI = 0.24–0.99), and lower in the interval from winter 2014 to summer 2014 (S_M = 0.59, 95% CI = 0.11–0.94; S_F = 0.67, 95% CI = 0.16–0.96). Estimates of kit fox density from SECR models were similar across sessions (0.018–0.022 animals/km^2); these estimates were among the lowest reported in the literature and at DPG. Derived estimates of kit fox abundance from SECR models were generally higher than those from robust design non-spatial models. The model-averaged abundance estimates from robust design non-spatial models indicated that there were 60.1–73.2 kit foxes in the study area and 95% confidence intervals suggested that population abundance was stable across sessions. Naïve estimates of coyote occupancy were >0.7 in all but the first session and probability of occurrence was not significantly different from 1. For kit foxes, naïve estimates of occupancy were ≤0.3, with the probability of occurrence estimated to be ≤0.5. Coyote occupancy was unrelated to water availability, but was positively related to the proportion of shrubland and woodland habitat. Kit fox occupancy displayed an inverse relationship, being negatively related to shrubland and woodland habitat. Kit fox probability of local extinction was positively related to site-level coyote activity, and within an occupied site, the probability of kit fox detection was positively related to transect-level coyote activity.

We estimated abundance for Sonoran pronghorn in 2013 and 2014 and annual survival between 2013 and 2014. The population using developed water holes (drinkers) was 116 (95% CI: 102–131) and 121 (95% CI: 112–132) in 2013 and 2014. The combined population estimate for drinker and non-drinker locations was 144 (95% CI: 132–157). Adults had higher annual survival probabilities (0.83, 95% CI: 0.69–0.92) than fawns (0.41, 95% CI: 0.21–0.65). Simulations were used to evaluate empirical estimates and evaluate study design tradeoffs. Our simulation results indicate our empirical estimates are reliable. Cost per individual monitored in 2014 was ~$184 USD for NGS-CR methods and $599 USD for aerial sightability methods. However, our results indicate that at the current estimated abundance (~200), the same level of precision (aerial CV ~ 21%) can be obtained using NGS-CR methods for ~$5,800, or an annual cost savings of over $4,000 (Woodruff et al. In Review).

IMPLEMENTATION ISSUES

In transferring this technology to other installations, we see the following challenges: 1) unpredictable weather and land access limitations can lead to insufficient sampling; 2) laboratories that can do these analyses need to be identified; and 3) experts will need to be identified to conduct quantitative analyses if the necessary expertise is not present within the DoD management team at the implementing installation.

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1.0 INTRODUCTION

1.1 BACKGROUND

Lands managed by the Department of Defense (DoD) support the greatest densities of species of conservation concern among lands operated by federal agencies (Stein et al. 2008). Under the provisions of the Endangered Species Act (ESA) and the Sikes Act, the DoD is challenged to protect and provide for the conservation of these species, while adhering to the military mission. Thus, the DoD needs reliable, accurate, and cost-effective methods for monitoring populations, while minimizing impacts on military training. Managers require methods for estimating population distribution, abundance, survival, reproduction, movements, and genetic diversity.

Many species are notoriously difficult to monitor because they are wide-ranging, occur in low densities, or are otherwise not amenable to traditional methods. Many common approaches (e.g., telemetry, capture-recapture, aerial surveys) for population monitoring rely on visual observation and physical capture of animals, are difficult, and can be expensive and time consuming. Other less invasive methods (e.g., scent-stations, spotlighting, scat surveys) may be more practical, but often provide only indices of abundance, neglecting information on true abundance, survival, reproduction, and movements (Long et al. 2008). Thus, there is great potential for DoD to benefit from implementing the most effective and innovative approaches for monitoring populations.

Noninvasive genetic sampling (NGS) can be combined with capture-recapture methods (NGS-CR) to accurately and efficiently monitor populations (e.g., abundance, survival). With NGS-CR, populations can be inventoried and monitored by collecting hair, feces, or feathers, without the need to disturb, capture, or even see the animals (Taberlet et al. 1999, Beja-Pereira et al. 2009). NGS-CR monitoring often can be completed at reduced costs relative to live-capture monitoring (e.g., Stenglein et al. 2010a). Additionally, under an appropriate spatio-temporal sampling design, NGS can be combined with occupancy modeling (NGS-OM) to efficiently and accurately estimate occupancy parameters. Although NGS-CR requires individual identification of noninvasive samples, NGS-OM requires only species identification, reducing laboratory costs. Furthermore, while NGS-CR provides information on parameters such as abundance and survival, NGS-OM offers insights into the spatial dynamics of population by estimating the probabilities of occupancy, colonization, and local extinction (MacKenzie et al. 2006).

1.2 OBJECTIVE OF THE DEMONSTRATION

The primary goal of this project was to demonstrate how NGS could be combined with capture-recapture modeling to evaluate the status of species of concern and their responses to management or military activities. We evaluated NGS-CR as a viable, long-term monitoring approach for species on DoD installations by comparing the cost-benefit with alternative approaches. We implemented monitoring for two species with different sampling challenges: the kit fox (*Vulpes macrotis*), a species of concern for western installations, and the Sonoran pronghorn (*Antilocapra americana sonoriensis*), an endangered subspecies that occurs in Arizona. For each species, we developed a spatio-temporal sampling design for acquiring noninvasive genetic data (via scats) from individuals, genotyped samples for individual ID, analyzed genotypes with capture-recapture methods to estimate population parameters, and developed protocols for long-term monitoring. We quantified expenditures and performed a cost-benefit analysis.

Specific objectives were to: (1) develop and implement spatio-temporal sampling designs for collection of fecal samples for use within a capture-recapture model, (2) quantify variation in estimates of population parameters and perform power analyses to determine the sampling effort required to achieve desired levels of precision, (3) develop long-term NGS-CR monitoring protocols, (4) compare cost-benefit of monitoring populations using NGS-CR versus alternative methods, and (5) facilitate transference to other species of concern on DoD installations for which NGS-CR approaches would be a preferred alternative. All objectives were accomplished.

A secondary goal was to demonstrate how NGS-OM could be used as an alternative monitoring strategy. NGS-OM can be implemented at reduced costs relative to NGS-CR and provides data on species occurrence, spatial dynamics, and species-habitat and interspecific interactions. We focused on kit foxes and their sympatric intraguild predator, coyotes (*Canis latrans*) at Dugway Proving Ground (DPG). We developed a sampling design that allowed concurrent monitoring of these species, used genetic ID to confirm species (De Barba et al. 2014), and employed dynamic occupancy models to estimate probabilities of detection, occupancy, local extinction, and colonization, while assessing the influences of coyotes and environmental covariates on kit fox space-use (MacKenzie et al. 2006). We compared costs with NGS-CR monitoring. The approach was successful and our objectives of providing a cost-effective alternative were met.

1.3 REGULATORY DRIVERS

Under the Sikes Act U.S. military installations are required to prepare, implement, review, and revise Integrated Natural Resource Management Plans (INRMPs) to provide for conservation of species at risk and other natural resources. The ESA requires federal agencies to not jeopardize the persistence of endangered or threatened species. Other DoD guidance related to management of listed species and biological diversity include DoD Instruction 4715.03, Army Regulation 200-3, U.S. Air Force Instruction 32-7064, U.S. Navy Instruction OPNAVINST 5090.1B, and U.S. Marine Corps Order MCO-P5090.2A.

2.0 TECHNOLOGY/METHODOLOGY DESCRIPTION

2.1 TECHNOLOGY/METHODOLOGY OVERVIEW

2.1.1 NGS-CR Technology Description

Capture-recapture modeling (Williams et al. 2002) has been commonly used to estimate parameters for wild populations. Based on modeling initial capture and recapture probabilities of individuals as a function of population size, the approach was first used to estimate wildlife population abundance by Lincoln (1930). Since, methods have been advanced to estimate other parameters such as survival, reproduction, and dispersal. The process involves capturing individuals and marking them so that they can be distinguished on subsequent capture occasions.

In capture-recapture modeling, the sampling design is described by the frequency of capture occasions. Pollock's robust design (Pollock et al. 1990, Kendall et al. 1997), consists of two types of periods. Primary sampling periods are separated by relatively long time intervals (e.g., several months, years); within primary periods, secondary sampling periods are separated by relatively short intervals (e.g., days, weeks). Populations are assumed to be demographically and geographically closed within, and open between, primary periods. The robust design allows for estimation of abundance within primary periods, survival, and temporary emigration between primary periods, and capture probability during secondary periods (Figure 1).

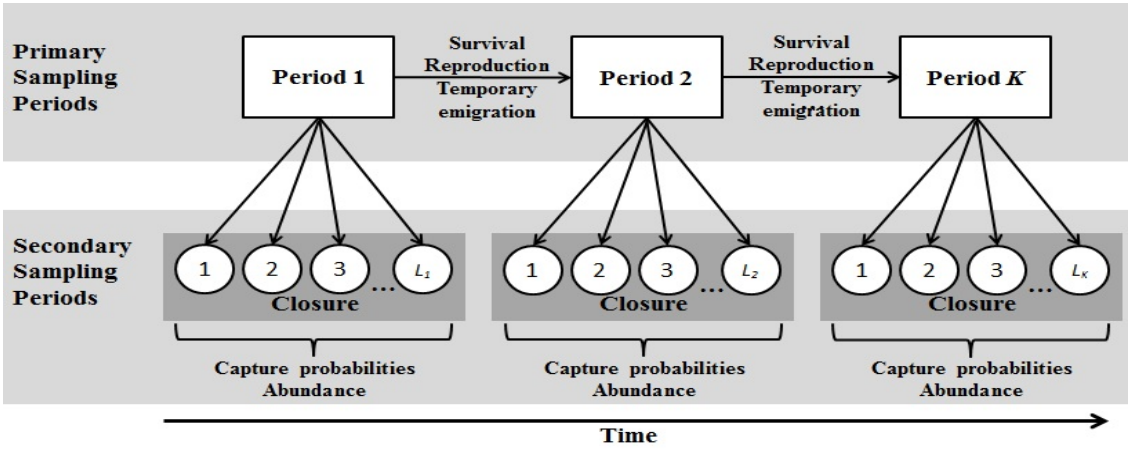

Figure 1. Pollock's Robust Sampling Design for Capture-Recapture Analyses.

Data collection under the robust design allows for estimation under alternative capture-recapture models as well, such as Cormack-Jolly-Seber and several single-session estimators of abundance (Lukacs and Burnham 2005, Miller et al. 2005, Puechmaille and Petit 2007).

NGS was first used to obtain genetic samples from brown bears (*Ursus arctos*; Höss et al. 1992, Taberlet and Bouvet 1992) and chimpanzees (*Pan troglodytes;* Morin and Woodruff 1992*).* Since, researchers have used NGS for rare species detection, abundance estimation, forensic applications, and to study hybridization, gene flow, predation, diets, and mating systems (Beja-Pereira et al. 2009). NGS-CR is appealing because noninvasive DNA sources (e.g., scat, hair) can be used for individual ID without having to catch, handle, or observe individuals, and can be more cost-effective than traditional capture methods (De Barba et al. 2010, Stenglein et al. 2010a). Encounters of unique

genotypes can be used in capture-recapture models and to conduct population viability analyses (Lukacs and Burnham 2005, Petit and Valiere 2005). Figure 2 provides an overview of the NGS-CR demonstration.

2.1.2 NGS-OM Technology Description

Species tend to be distributed in association with resources, but can be restricted by competition and/or predation. Knowledge of how habitat and interspecific interactions drive space-use by species can improve management policies. Occupancy modeling uses repeated surveys to account for imperfect detection and produce unbiased parameter estimates (MacKenzie et al. 2006). The unit of analysis in occupancy studies is the survey site, allowing occurrence to be modeled as a function of site characteristics (e.g., habitat). Recent advances in multi-species occupancy models (i.e., co-occurrence models; Richmond et al. 2010) and dynamic occupancy models (i.e., multi-season models; MacKenzie et al. 2003) have allowed practitioners to investigate species interactions and dynamic processes (i.e., local colonization and extinction). While single-season occupancy modeling has become a common monitoring strategy for wildlife populations, these only provide estimates for probabilities of detection and occurrence. Dynamic models allow practitioners to formally assess patterns of colonization and local extinction (MacKenzie et al. 2003). Like robust design for capture-recapture (Figure 1), dynamic occupancy models consist of primary and secondary periods. Sampling sites are assumed to be closed to changes in occupancy within, and open between, primary periods.

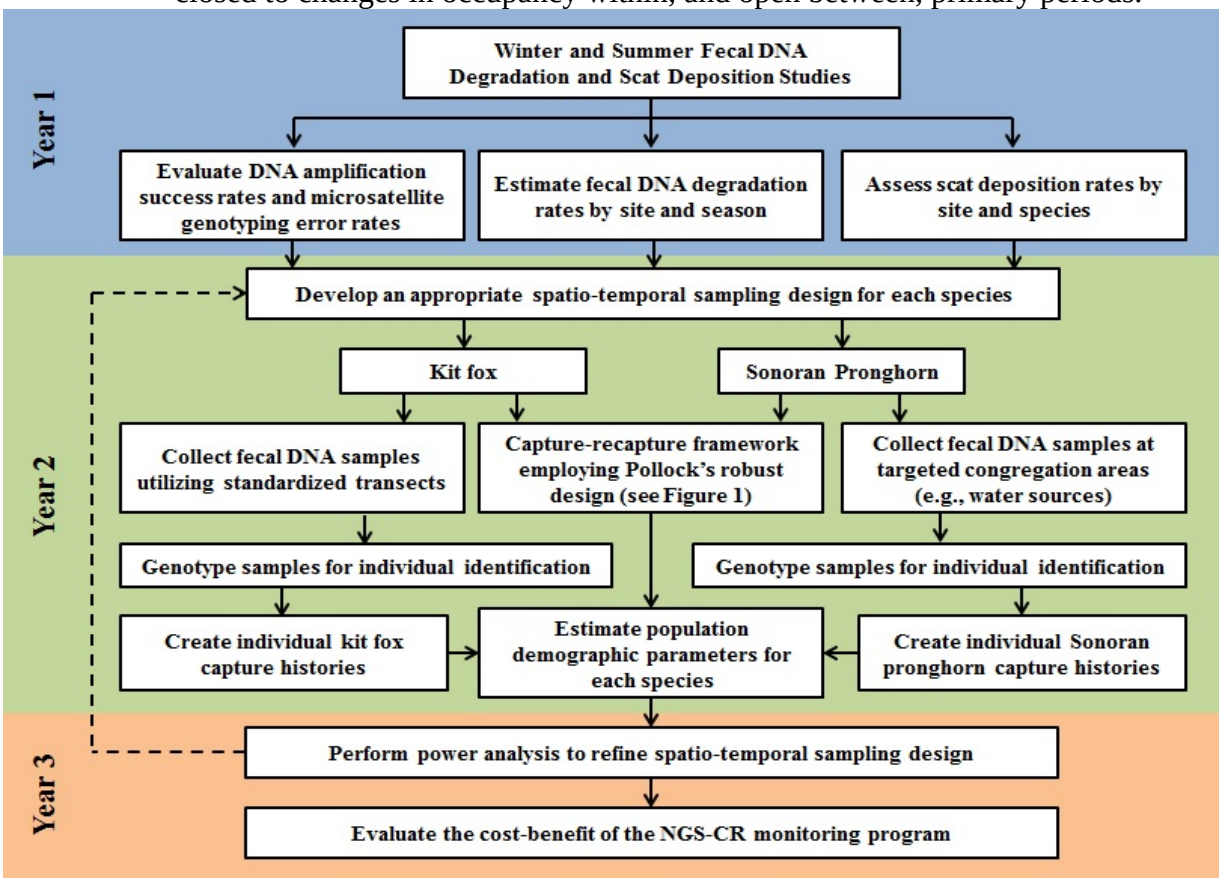

Figure 2. Flow Diagram of the Demonstration's Technology and Methodology.

2.1.3 Expected Applications

This project will benefit DPG by providing information needed to manage kit foxes and evaluate effects of military actions on populations. These efforts will assist managers in proactively preventing kit foxes from being listed under the ESA. For Sonoran pronghorn, there are substantial costs to DoD due to their ESA status (McCullough 2005). To be downlisted, it will be necessary to have reliable data from the re-established populations. Furthermore, this project offers guidance for monitoring other populations of concern. The standardized transect sampling approach could be used to monitor carnivores across many installations (see Section 8.0).

2.2 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

2.2.1 Noninvasive Genetic Sampling Capture-Recapture (NGS-CR)

NGS-CR has the potential to provide reliable estimates of population parameters at reduced costs and with minimal stress to animals (Waits and Paetkau 2005, Luikart et al. 2010). Additionally, NGS-CR provides genetic data that can be used to evaluate other measures of population health including genetic diversity, population connectivity, and effective population size (N_e).

One challenge of NGS-CR is data quality, which is affected by DNA degradation and genotyping errors (Taberlet et al. 1999, Waits and Paetkau 2005). Numerous studies indicate DNA degradation and genotyping errors vary among species and environmental conditions. Sampling the freshest scats and conducting surveys during the driest and/or coldest seasons can reduce degradation and genotyping errors (Murphy et al. 2007, Santini et al. 2007). Improved laboratory techniques and estimation models continually improve our ability to limit the effects of genotyping errors (e.g. Wright et al. 2009, Beja-Pereira et al. 2009).

Reliable parameter estimates from capture-recapture models require sufficient sample sizes, capture probabilities and low capture biases. For NGS-CR, sample availability is influenced by sample deposition and removal rates which can vary spatially and temporally. Our Year 1 pilot study was designed to estimate genotyping success, error rates, sample sizes, and capture probabilities to determine the optimal sampling design for subsequent years via simulations.

2.2.2 Noninvasive Genetic Sampling Occupancy Modeling (NGS-OM)

NGS-OM offers an efficient framework to investigate the spatial distribution and dynamics of species (MacKenzie et al. 2006). NGS-OM requires only species ID, making it more cost-effective than NGS-CR. NGS-OM may fail to detect changes in abundance, particularly with territorial species which may experience declines in abundance with little change in occupancy. Still, occupancy offers insights into the processes driving space-use and potential impacts that military activities on local extinction and/or colonization. Occupancy can be implemented within an NGS-CR design, so long as the sampling design accommodates both approaches.

Parameter estimation within an occupancy modeling framework assumes no misidentification of species, which can severely bias results (MacKenzie et al. 2006). Field-based ID of carnivore scats can suffer from high misclassification rates (Lonsinger et al. 2015b). Incorporating genetic species

ID into NGS-OM can minimize misidentification error. Nonparametric classification based on morphometrics can reduce misidentifications over field ID (Lonsinger et al. 2015b).

2.2.3 Species and Site-specific Limitations

Both NGS-CR and NGS-OM approaches for kit fox may be influenced by weather conditions. Heavy snowfall can preserve samples, but also may make scats unavailable for detection until snow melts; this delayed availability may influence closure assumptions, particularly if scats are detected long after deposition and still successfully amplify. NGS conducted along roads can increase detection for carnivores over alternative strategies (Schauster et al. 2002, Dempsey et al. 2014), but is restricted to sites with sufficient road coverage. Vehicle traffic can significantly increase scat removal, which may vary spatially and temporally (Lonsinger et al. 2016).

For pronghorn, the timing of sampling may vary annually with changing weather and forage conditions. Lower drinker visitation results in lower detection probabilities and these differences need to be considered when monitoring trends. Due to our targeted sampling, our estimates apply only to the individuals using drinkers. Twice as many males have been released from the captive pen, potentially leading to a male bias at drinkers (United States Fish and Wildlife Service [USFWS] 2015). Additionally, home range size and movement rates likely differ between sexes (Ockenfels et al. 1994, Clemente et al. 1995) which could affect use and representation of sexes at drinkers. Thus, any extrapolation to the entire population, especially pertaining to sex ratios, should include a correction factor.

3.0 PERFORMANCE OBJECTIVES

Table 1. Performance Objectives

Performance Objective	Metric	Data Requirements	Success Criteria	Results
Quantitative Performance Objectives				
1. Improved monitoring protocol for kit fox and Sonoran pronghorn based on NGS-CR	• Increase in the number of demographic parameters reliably estimated versus alternatives	• Number of demographic parameters that can be reliably estimated via NGS-CR	• Number of parameters obtained via NGS-CR is > number obtained from current approaches	• Kit fox: Yes • Pronghorn: Yes
2. Obtain reliable estimates of demographic parameters via implementation of NGS-CR monitoring protocol (kit fox and Sonoran pronghorn)	• Measures of precision for estimates of 1. Abundance 2. Survival 3. Reproduction 4. Population connectivity 5. Genetic diversity	• Mean values of parameter estimates • Estimates of precision (e.g., standard error) for parameter estimates	• "Reliable" estimates for abundance are those with a coefficient of variation <10% • For the other parameters, we evaluated only if it was possible to obtain the estimate from the available data	<u>Abundance and Survival</u> • Kit fox: Yes • Pronghorn: Yes <u>Reproduction</u> • Kit fox: No • Pronghorn: Yes <u>Connectivity and Diversity</u> • Kit fox: Yes • Pronghorn: Yes
3. Improve efficiency of current monitoring programs	• Increased cost-benefit of monitoring programs • Increase in the spatial extent of area monitored versus alternatives • Increase in the temporal resolution of estimates versus alternatives	• Sampling design (i.e., area sampled, frequency and quantity of scat collection) • Spatial area of inference for monitoring program • Temporal extent and resolution of monitoring program • Number of reliable parameters that can be obtained via NGS-CR versus alternatives • Cost of obtaining parameters via NGS-CR versus alternatives	• The cost of obtaining each parameter based on NGS-CR is < cost of alternatives • The sum cost of obtaining all parameters based on NGS-CR is < cost of alternatives • Given a fixed cost for monitoring, area monitored is $\geq$ the area that could be monitored under current approaches • Given a fixed cost for monitoring, estimates of demographic parameters can be obtained more often than current approaches	• Cost < pronghorn • Increased spatial extent for kit fox • Increased temporal frequency for pronghorn
4. Ease of use	• Ability of a technician-level individual to implement sampling design	• Feedback from technicians on ease of data collection via standard Likert survey (1= strongly disagree to 5= strongly agree)	• Responses from Likert survey indicates agreement with ease of implementation of field protocol by a score ≥3.5.	• Yes

Table 1. Performance Objectives (Continued)

Performance Objective	Metric	Data Requirements	Success Criteria	Results
5. Obtain estimates of occupancy and dynamic parameters via NGS-OM monitoring for kit foxes, at reduced costs relative to NGS-CR	• Parameter estimates: 1. Proportion of area occupied 2. Colonization 3. Extinction 4. Species interactions 5. Costs	• Parameter estimates • Costs of implementation	• Effective parameter estimates and inferences on species interactions • Reduced cost relative alternative monitoring strategies	• Yes
Qualitative Performance Objectives				
6. Implementation of monitoring programs for kit fox and Sonoran pronghorn based on NGS-CR	• Consideration of implementing a NGS-CR monitoring program by federal/ state agencies or other organizations	• Records of interactions with persons responsible for managing focal species regarding implementation of monitoring programs based on NGS-CR	• Demonstrated interest and positive interactions about NGS-CR monitoring from federal/ state agencies or other organizations and/or a Likert score ≥3.5.	• Yes

4.0 SITE DESCRIPTION

4.1 SITE LOCATION AND HISTORY

We implemented monitoring programs based on NGS-CR for kit foxes on DPG in Utah (Figure 3) and Sonoran pronghorn on Barry M. Goldwater Range (BMGR) in Arizona (Figure 4).

4.2 SITE CHARACTERISTICS

4.2.1 Dugway Proving Ground (DPG), Utah

Located in the Great Basin, habitats at DPG include Cold Desert Playa and Chenopod Shrubland, vegetated and unvegetated dunes, non-native invasive grasslands, greasewood and sagebrush shrublands, and open juniper woodlands; wetland habitats are available to a lesser extent (DPG 2007). Elevations range from 1228 to 2154 m (DPG 2007). DPG experiences cold winters (January: average high = 4°C, average low = -10°C), moderate summers (July: average high = 36°C, average low = 15°C), and limited rainfall (average annual ~ 21.9 cm; DPG 2007). Water was historically scarce (Arjo et al. 2007). Four sewage lagoons, 11 wildlife guzzlers, collection ponds, livestock tanks (on neighboring lands), and irrigation have increased water availability. Among carnivores at DPG, coyotes and red foxes compete most directly with kit foxes. Coyote populations have increased since the 1950's when researchers indicated that they were rare (Arjo et al. 2007). Red foxes have been detected at DPG, but are suspected to be rare. The habitat requirements of native felids overlap to a much lesser extent with kit foxes. We detected kit foxes, coyotes, red foxes, bobcats, mountain lions, and domestic dogs during the demonstration.

4.2.2 Barry M. Goldwater Range and Cabeza Prieta National Wildlife Refuge, Arizona

The BMGR and Cabeza Prieta National Wildlife Refuge (CPNWR), in southwestern Arizona, are in one of the hottest and driest regions of North America. Summer temperatures can be above 32–38 °C for >100 days in a row (USFWS 2011). Average annual precipitation is <21.7 cm (INRMP 2003). Artificial drinkers provide water to pronghorn and other species, and supplemental feed is provided at some sites from April to November (USFWS 2015). Forage enhancement plots promote growth of natural vegetation. Pronghorn are attracted to military use areas due to pooling of water in bomb craters, ease of predator detection, and increased forage due to disturbance and fires (Hervert et al. 1997, Krausman et al. 2005). Biologists conduct visual scans for pronghorn prior to bombing missions. If pronghorn are within 5 km of a target, the mission is called off or redirected (Luke Air Force Base [LAFB] 2012). A semi-captive breeding pen facilitates pronghorn recovery (Otte 2006) through release of captive animals. Since 2006, 128 radio-collared pronghorn have been released from the pen (USFWS 2015). Monitoring of wild pronghorn consists of locating radio-collared individuals aerially (bimonthly) and from the ground and a biennial population count (USFWS 2015).

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5.0 TEST DESIGN

5.1 CONCEPTUAL TEST DESIGN

The demonstration included four stages. Stage 1 included (1) evaluation of fecal deposition rates, (2) development of species and individual ID methods, and (3) DNA degradation experiments. Stage 2 included implementation of NGS-CR population estimation methods and power analyses to determine the optimal spatio-temporal sampling design. Stage 3 implemented the optimal sampling designs. Stage 4 involved cost-benefit analyses of NGS-CR approaches methods.

We conducted sampling under a robust design and collected data on implementation costs. We explored ways to further reduce costs by assessing the effectiveness of subsampling, evaluating new NGS-CR models to reduce costs, and employing power analyses to evaluate effort required to achieve desired precision. In addition to the non-spatial robust design and 'capture with replacement' (CAPWIRE) models, we evaluated spatially-explicit models for kit foxes. For kit foxes, we also demonstrated the utility of NGS-OM, including how spatial replication can be used to increase the spatial extent of monitoring and investigate the influence of interspecific interactions when co-occurrence modeling was impractical. We also evaluated (1) statistical classification approaches for scat ID, and (2) rates of scat removal, to further improve efficiency.

5.2 BASELINE CHARACTERIZATION AND PREPARATION

For kit foxes, we chose DPG as our focal installation to leverage concurrent research (E. Gese, Utah State University [USU]) that could provide alternative estimates of abundance, distribution, and survival based on live-capture and telemetry monitoring (Arjo et al. 2007, Kozlowski et al. 2008). Scat deposition surveys conducted by Dr. Gese provided baseline data on scat encounter rates. Also, during our 2012 and 2013 field seasons, Dr. Gese tracked ~25 telemetered kit foxes on DPG.

Sonoran pronghorn have been federally listed as endangered since 1967 (USFWS 2015). Most of the US population resides on the southwestern portion of BMGR and CPNWR. Sonoran pronghorn use anthropogenic water sources (i.e., drinkers), especially during drier periods (Morgart et al. 2005). The USFWS maintains a captive pronghorn population, which we utilized for our degradation study. We used Deoxyribonucleic Acid (DNA) samples (blood) collected by Melanie Culver (University of Arizona) from known age individuals during annual capture operations to distinguish age class from morphometric measurements of fecal pellets.

5.3 DESIGN AND LAYOUT OF TECHNOLOGY AND METHODOLOGY COMPONENTS

5.3.1 Laboratory Methodology for DNA Extraction, Species ID, and Individual ID

DNA was extracted from fecal samples using the QIAamp DNA Stool Mini Kit (Qiagen, Inc.). Species-specific polymerase chain reaction (PCR) primers were designed to conduct species ID. PCR procedures were conducted on a BioRad Tetrad. We had already developed methods to identify kit fox DNA using a mitochondrial DNA fragment analysis test (De Barba et al. 2014). We designed a similar test to distinguish pronghorn pellets from mule deer (Woodruff et al. 2014). Fragment analysis and sequencing products were visualized using a 3130xl DNA Sequencer and alleles were scored using Genemapper 3.7 (Applied Biosystems).

Individual and sex ID were conducted using nuclear DNA microsatellite analysis at 6–9 loci for kit foxes and 7–10 loci for pronghorn plus a sex ID primer for each species. The PCR products were separated by size using the same methods as species ID. Low quality samples were culled. For consensus genotypes, we required an identical result across two PCRs for heterozygotes and three for homozygotes. Completed genotypes were analyzed using the software GenAlEx6 (Peakall and Smouse 2001) or the ConGenR script (Lonsinger and Waits 2015). All laboratory analyses were conducted at the Laboratory for Ecological, Evolutionary, and Conservation Genetics at the University of Idaho. This facility is directed by PI Waits.

5.4 FIELD TESTING

We evaluated fecal deposition and DNA degradation rates for each species, allowing us to predict the number of scats that would be deposited per unit area per unit time, and the proportion of these that would be expected to provide reliable genotypes. This information was essential to identifying appropriate spatio-temporal sampling designs in year 2 and year 3.

We implemented kit fox monitoring during two primary periods, winter and summer, which aligned with periods preceding breeding and juvenile dispersal, respectively. The number of NGS-CR secondary periods (or occasions), and the duration of time between occasions was informed by our pilot study (Lonsinger et al. 2015a) and power analyses. In year 2, the duration of time between surveys of a transect was informed by our optimization scheme (Lonsinger et al. 2015a). For year 3, we adjusted the number of occasions based on the results of power analyses.

For pronghorn, we implemented a single primary sampling period in May and June each year. Timing coincided with the hot dry months when pronghorns were using feed and water sites. Our pilot studies (Woodruff et al. 2014, 2015) informed the number and sampling interval for secondary sampling periods. In Year 2, we collected feces during 3 secondary periods separated by 7-day intervals during 2 primary periods (May–June and October–November). A power analysis conducted to inform year 3 sampling to achieve a CV <10% in the parameter estimates. Thus, we did not change the sampling design in Year 3; however, we did sample during only a single primary period (June) due to the paucity of samples collected during the fall in Year 2.

5.5 SAMPLING PROTOCOL

5.5.1 Dugway Proving Ground, Utah

Sampling occurred during two winter (January to March) and two summer (July and August) primary periods (sessions), during 2013 and 2014. We employed two sampling designs for kit foxes. The first, used a robust sampling design, in which sampling occurred along thirty 5 km multi-occasion transects (each surveyed 3–5 times [occasions] per session; i.e., temporal replication; Figure 3). The time between occasions was ~14 days. Additionally, we surveyed each of the four single-occasion 500 m transects (within each of the 60 [6.25 km^2] sites selected for NGS-OM) once per session (Figure 3). The estimated effective sampling area was 3,663 km^2 (Figure 3). Each transect followed dirt or gravel roads and were surveyed for carnivore scats. From scats we collected ~0.7 ml of fecal material into 1.4 ml of DETs buffer (Seutin et al. 1991). We performed species ID tests on all samples and culled low quality samples. For individual ID, we performed up to eight replicates per sample to establish consensus genotypes, evaluating amplification success and genotyping error rates with ConGenR (Lonsinger and Waits 2015).

Encounter histories for NGS-CR analyses included all samples from multi-occasion and single-occasion transects. For NGS-OM analyses, encounter histories included samples from single-occasion transects, as well as scats detected during the first sampling occasion at 43 additional sites (6.25 km^2) that contained ≥2 km of the 30 multi-occasion transects. We analyzed kit fox capture data with (i) non-spatial Huggins closed-capture models (Huggins 1989), (ii) Spatially Explicit Capture-Recapture (SECR) models (Borchers and Efford 2008), and (iii) CAPWIRE models (Miller et al. 2005). Huggins models were fit using a robust design framework (Huggins 1989, Pollock et al. 1990) in program MARK (White and Burnham 1999) and provided estimates of apparent survival probability (*S*), capture probability (*p*), and recapture probability (*c*), as well as inferences regarding temporary immigration (1 – γ") and temporary emigration (γ'). We used Akaike's Information Criterion with small sample size correction (AICc) and Akaike weights to compare relative model fit (Burnham and Anderson 2002). Parameter estimates accounting for model-selection uncertainty were achieved by model-averaging (Burnham and Anderson 2002), with associated variances and confidence intervals (CIs) being generated with the delta method (Williams et al. 2002).

We fit SECR models with the full likelihood using a multi-session formulation and half-normal detection function (Efford et al. 2009) with the 'secr' package (Efford 2015; R Core Team 2015). SECR models provided estimates of capture probability (defined as two parameters, detection function intercept [*g0*] and detection function scale parameter [σ]) and density (*D*); we identified an appropriate effective sampling area (Figure 3) to derive an estimate of abundance (i.e., *D* * effective sampling area). We used AICc and Akaike weights (Burnham and Anderson 2002) to compare the relative fit of models.

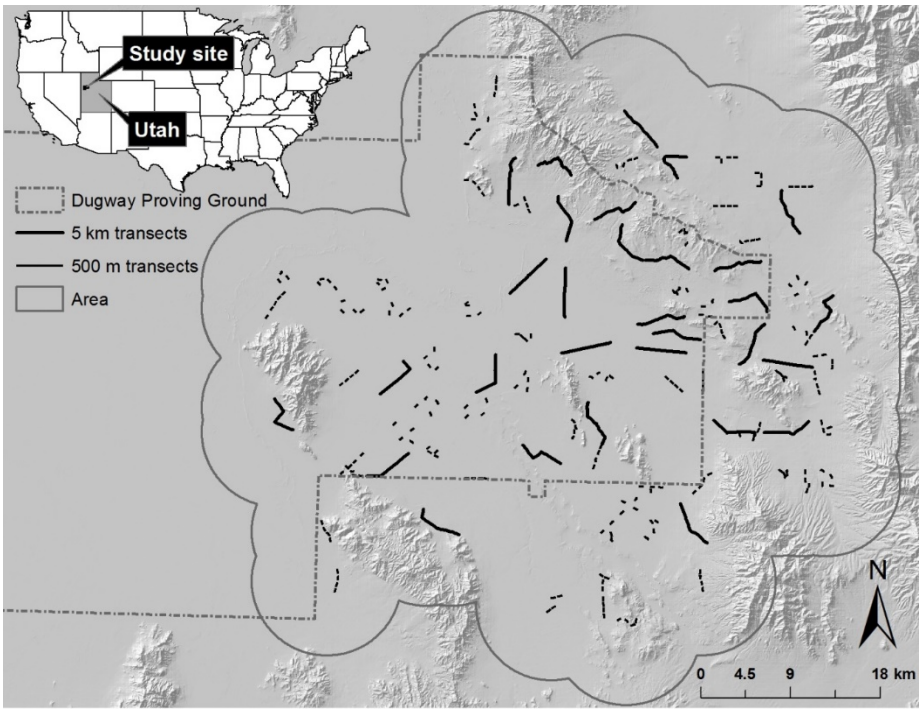

Figure 3. Location of 5 km Multi-Occasion and 500 m Single-occasion Transects Surveyed for Kit Fox Scats, 2013–2014.

Area represents the effective sampling area.

For CAPWIRE, we fit models for each session independently with the 'capwire' package (Pennell et al. 2013, R Core Team 2015). We fit both single-occasion and multi-occasion formulations of the equal capture and two-innate rate models each session, and compared model fit using a likelihood-ratio test. We generated 95% CIs for the estimate of the best supported model using parametric bootstraps (Miller et al. 2005, Pennell et al. 2013).

For occupancy, we used dynamic single-species occupancy models for kit foxes that included both environmental covariates and indices of coyote activity, exploiting the variation in coyote activity. We used a multi-stage approach within program MARK (White and Burnham 1999). Environmental covariates were obtained from available Geographic Information System (GIS) layers. Indices of coyote activity were based on the number of coyote scats detected during surveys. We sequentially fit models for probabilities of detection (p), occupancy (ψ), and the dynamic parameters (local extinction [ε] and colonization [γ]) together. For each model set, we used AICc to compare models and cumulative Akaike weights to evaluate predictor importance (Burnham and Anderson 2002).

5.5.2 Barry M. Goldwater Range and Cabeza Prieta National Wildlife Refuge (NWR), Arizona

In May and June 2013 and 2014, we attempted to collect fecal samples three times (six total) at an interval of seven days at all drinkers likely to be used by pronghorn (17) (Figure 4, Woodruff et al. 2016b). In 2014 we collected samples from nine groups located away from (>1 km) drinkers. For each sampling event, we collected samples within 50 m of drinkers (Woodruff et al. 2015) at a rate of three times the number of pronghorn at the drinker. Samples were field-classified based on visual inspection of morphology as adult (≥1 year old) or fawn (<1 year old). We analyzed ~2 samples per pronghorn, starting with the freshest samples. Genotyping errors were calculated following Broquet and Petit (2004) using ConGenR (Lonsinger and Waits 2015).

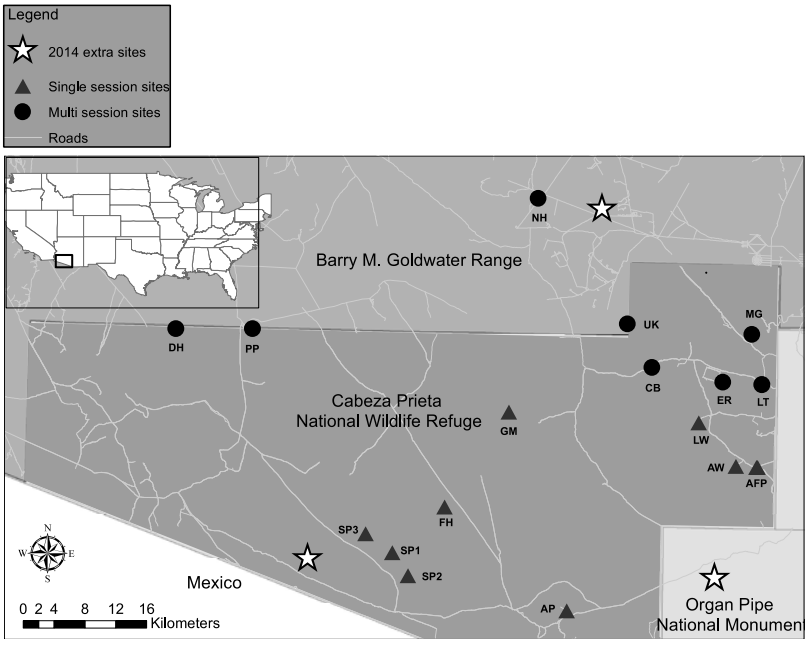

Figure 4. Study Area and Location of Sampling Sites on Barry M. Goldwater Range and Cabeza Prieta NWR for Noninvasive Genetic Sampling of Sonoran Pronghorn.

Abundance and Survival Estimation

We generated an encounter history for each individual counting only a single detection per individual per sampling session. Adult and fawn models assumed equal detection and redetection probability. We used AIC_c to evaluate relative support for each model and model averaged parameter estimates and standard errors over all models (Burnham & Anderson 2002).

For adults, we applied Huggins' robust design in the development of four models of survival and abundance in Program MARK (White and Burnham 1999). We estimated capture/detection (*p*) and recapture/redetection probabilities (*c*), abundance (*N*), and annual apparent survival. We performed analyses on drinker locations only from 2013 and 2014, as well as on all locations (drinker and non-drinker) in 2014. For fawns, we used closed capture models in each year to estimate *c*, *p,* and *N* 2013 and 2014, again, modeling drinker only and all locations separately. We used a full likelihood robust design model to estimate annual apparent survival for the 2013 fawn cohort. We summed population estimates for adult male, adult female, and fawn and calculated standard errors for abundance using the Delta method (Seber 1982).

Comparing Abundance Estimators and Simulations

To evaluate precision under reduced sampling efforts, we analyzed our data (1) with a reduced number of secondary periods per primary period and the number of samples analyzed per secondary period; (2) by reanalyzing the data using single-session (*capwire*) models; and (3) using simulations to estimate the optimal number of consensus genotypes needed for precise abundance estimates (CV≤10–20%; Pollock et al.1990). We evaluated each sampling design and estimator by comparing the simulated abundance estimates to the true population size (percent bias), the CV, the relative mean squared error (RMSE), and the 95% CI coverage (proportion of times [out of 100] the true value was contained within the interval).

5.6 SAMPLING RESULTS

5.6.1 Dugway Proving Ground, Utah

Sampling, Genetic Analyses, and NGS-CR Modeling

We collected 3,752 carnivore scats, of which 21.6% and 63.3% of samples were determined to be kit fox and coyote, respectively. Six loci were required to identify individual kit foxes, excluding sex ID markers. Across sessions, we identified 109 kit foxes, with 36–50 being captured per session. From 103 sites per session, 1,702 samples contributed to NGS-OM. Soil for the majority of NGS-OM sites was predominantly silt or fine sand. Mean %SW for sites was 21.8% (± 2.25 SE). Distance to nearest water was 0.2–12.4 km (mean = 3.96 km ± 0.28 SE).

We compared the fit of 36 non-spatial Huggins models for kit fox *S* (Lonsinger 2015). Male kit fox survival (S_M) was slightly lower than female survival (S_F); annual S_M and S_F from summer 2013 to summer 2014 was 0.48 and 0.58, respectively, though estimates had poor precision. The best models suggested a trend in *p* within sessions (Lonsinger 2015). Model-averaged abundance from Huggins models suggested there were 60.1–73.2 kit foxes and that abundance was similar across sessions (Figure 5). Live-capture, radio-telemetry and den monitoring produced insufficient sample sizes to generate abundance estimates (B. Kluever, personal communication).

For SECR models, the top kit fox detection model included variation among sessions and a trend in capture parameters within sessions. We fit 14 models for kit fox density (Lonsinger 2015). The model $D \sim$ session received the most support and suggested D was similar across sessions (0.018–0.022 foxes/km^2). SECR estimates were similar to Huggins model estimates (Figure 5).

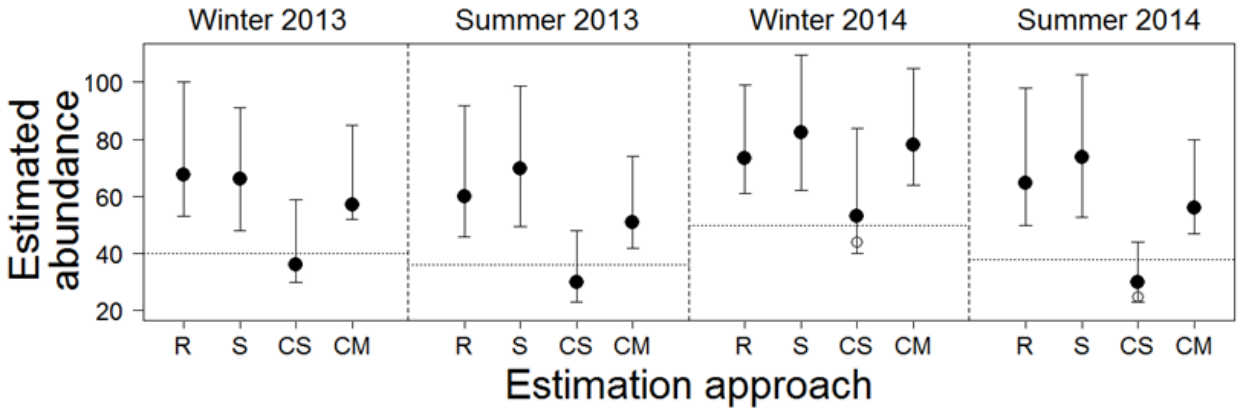

Figure 5. Estimated Abundances and 95% Confidence Intervals for Kit Foxes (*Vulpes macrotis*) in Western Utah over Four Sessions, 2013–2014.

Multiple estimators were used including robust design non-spatial Huggins closed-capture models (R), multi-session spatially explicit capture-recapture models (S), and two formulations of two-innate rates capture with replacement models based on single-occasion (CS) and multi-occasion (CM) sampling. Open circles represent capture with replacement point estimates under an equal capture model, where likelihood ratio tests failed to reject equal capture. The dashed horizontal line indicates the number of unique individuals identified within each session based on nuclear DNA.

When considering only transects contributing to single-occasion CAPWIRE models, we identified 21–30 kit foxes/session. Single-occasion estimates (30–53 kit foxes) were substantially lower than multi-session estimates, with 95% CIs that failed to overlap multi-session point estimates in three sessions (Figure 5). Multi-occasion CAPWIRE estimates were lower than multi-session estimates (except in winter 2014), but CIs overlapped considerably (Figure 5).

True abundance is unknown for our target populations and we cannot explicitly infer bias. Abundance estimates from multi-session Huggins and SECR models showed high levels of agreement (Figure 5). Non-spatial models do not account for 'holes' in the sampling frame (Williams et al. 2002, Efford and Fewster 2013), and this may contribute to the lower abundance estimates resulting from Huggins models. By accounting for proximity to animal activity centers, SECR models effectively handle holes (Borchers and Efford 2008, Royle et al. 2014).

We observed relatively consistent results and similar levels of precision between multi-session (i.e., Huggins and SECR) models, and we used these as a standard to evaluate the performance of single-occasion and multi-occasion CAPWIRE estimators. The minimum number known alive (MNKA) nearly always underestimates abundance (Mills et al. 2000); we regard estimates at or below the MNKA as biased. Single-occasion kit fox estimates fell below the MNKA for three sessions (all sessions when using the equal capture model where it was supported; Figure 5). The assumption of independence among captures may be violated when individuals are captured >1 time within a site.

While restricting recaptures to spatially disparate locations can reduce this concern (Stenglein et al. 2010b), we included all captures and this may have biased our results and inflated precision. Our sampling was relatively dispersed and temporal variation in space-use may limit the number of individuals available for capture during a single occasion, biasing CAPWIRE estimates (Kendall 1999). Combining captures from multiple occasions, while accounting for variable effort to meet model assumptions, allowed us to increase the number of initial captures, resulting in multi-occasion estimates that were closer to multi-session estimates.

NGS-OM Dynamic Models

Dynamic models for coyote occupancy and estimates are described in detail in Lonsinger (2015). In summary, coyote ψ was not significantly different than 1 across sessions. Thus, if kit fox occurred at a site, they co-occurred with coyotes. Kit fox ψ was substantially lower than coyote ψ. The best kit fox detection model suggested that kit fox *p* was positively related to transect-level coyote activity ($\beta = 0.20 \pm 0.06$ SE). The best kit fox occupancy model indicated %SW had a strong negative ($\beta = -13.46 \pm 3.98$ SE) influence on kit fox ψ; this was the inverse to the relationship between coyotes and %SW (Lonsinger 2015). The top model for kit fox dynamic parameters suggested that site-level coyote activity positively influenced ε ($\beta = 0.97 \pm 0.45$ SE) and soil type influenced γ (Lonsinger 2015). Derived estimates of kit fox ψ (<0.5) from the top model were similar across sessions. Kit fox ψ was less stable in sites with greater coyote activity.

Shrubland and woodland at DPG tended to support greater mammalian prey abundance and diversity than alternative habitats (Arjo et al.2007, Kozloswki et al. 2012) and may have provided greater thermal cover (Blaum et al. 2007). Coyote occupancy declined precipitously when %SW <20% (Lonsinger 2015); kit fox occupancy displayed an inverse relationship, suggesting broad scale habitat partitioning, aligning with finding of Kozlowski et al. (2012). Elucidating drivers of local extinction and colonization can improve our understanding of how covariates and species interactions drive space use (MacKenzie et al. 2003). As predicted, kit fox probability of local extinction was elevated across sites with higher coyote activity. Dietary overlap of coyotes and kit foxes was high at DPG (Kozlowski et al. 2008) and when sympatric, coyote predation accounts for a significant proportion of kit fox mortalities (e.g., Kozlowski et al. 2008). Thus, local extinction may result from a decreased ability to avoid intraguild predation at sites with higher coyote activity. Kit foxes utilize burrows year-round to provide relief from environmental conditions and predators (Arjo et al. 2003). Thus, it was not surprising that silty soil, which facilitates burrow excavation (Egoscue 1956), promoted kit fox colonization.

Co-occurrence among predators often requires subordinate species to adjust activity patterns or space use. At DPG, diets and nightly activity patterns of kit foxes and coyotes overlap (Kozlowski et al. 2008, 2012). Hall et al. (2013) supported the lack of temporal separation between species, but failed to detect spatial partitioning. The scale of inference is essential to understanding patterns of co-occurrence. Our results reconcile these conflicting results. At broad scales, coyote occupancy increased with increasing %SW, presumably reflecting resource matching, while kit fox occupancy demonstrated an inverse relationship reflecting patterns consistent with safety matching. At finer scales, there was a lack of spatial separation, with kit fox space use being highest where coyote activity was highest, suggesting that within their home ranges, kit foxes may use riskier habitats to secure sufficient resources (i.e., resource matching).

5.6.2 Barry M. Goldwater Range and Cabeza Prieta NWR, Arizona

From drinkers, we collected 730 (634 extracted) samples in 2013 and 980 (692 extracted) samples in 2014 and 79 non-drinker samples in 2014. At drinkers we detected 2.5–3 times more adult male than female samples. At drinkers, the population estimate was 116 individuals (95% CI: 101–132) in 2013 and 121 individuals (95% CI: 112–132) in 2014. For all locations (2014 only), the population estimate was 144 individuals (95% CI: 132–157).

Comparing Abundance Estimators and Simulations

Our empirical data had increased precision with more sessions. Population estimates changed only slightly with the inclusion of the extra samples. CMR and *capwire* gave similar population estimates and CIs substantially overlapped. In simulations, abundance was biased positively in *capwire* and negatively in CMR (Figure 6). High capture probabilities led to extremely precise estimates. Increasing sample size generally led to an improvement in bias and RMSE values for both estimators. Simulation results indicate our empirical estimates are reliable. We recommend collecting 1.5–2 samples/individual/session in ≥2 sessions and using a multi-session model.

NGS-CR costs totaled $18,512 and $20,271 in 2013 and 2014. The cost of the aerial survey is $10,000 annually (USFWS 2015). Cost per individual monitored using aerial methods was $92.59 and $59.52 in 2012 and 2014, respectively. If we match the cost of NGS-CR to the annual aerial monitoring expenditure ($10,000), for a true abundance of 200 individuals, we could obtain ~0.75 samples/individual/session over 2 sampling sessions. CV would be < 5% and RMSE would improve over a single session and would likely be better than RMSE from aerial estimates as well (Table 2). The cost of NGS became more expensive than aerial methods when improving RMSE to ≤0.5 which was possible only with population size ≤100.

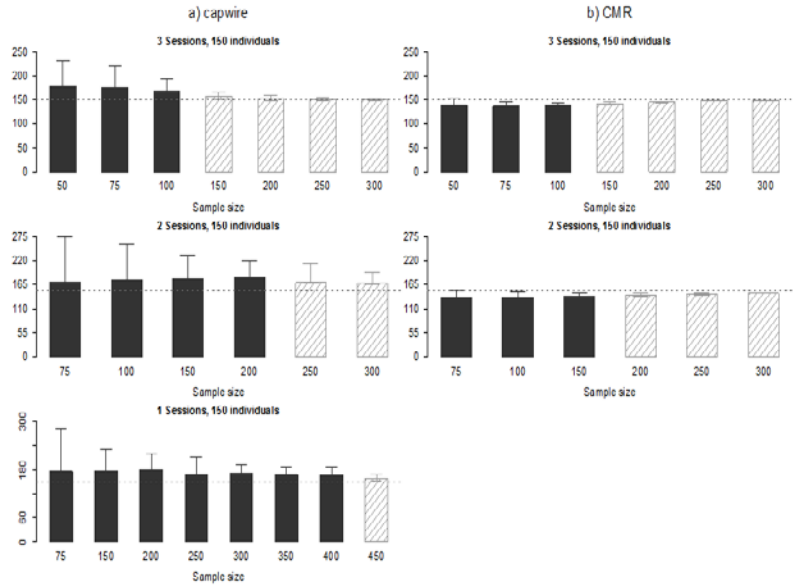

Figure 6. Abundance Estimates from Simulations for Sonoran Pronghorn with True Abundance 150 and 300 Individuals in 2 and 3 Sessions a) Single Session Models in *Capwire* and b) Multi Session Closed Capture (CMR) Models.

Solid color indicates relative mean squared error (RMSE) >0.5, and hashed represents RMSE ≤0.5. Not all results are shown, but trends were the same in all simulations.

6.0 PERFORMANCE ASSESSMENT

1. Demonstrate that monitoring programs for kit foxes and Sonoran pronghorn based on NGS-CR provides more information than currently implemented methods.

   We compared the total number of parameters that were or could be obtained to the number potentially obtained using currently implemented approaches. We considered this objective met if we were able to estimate a greater number of parameters than recent monitoring efforts. Our results demonstrated that we were able to estimate a greater number of population level parameters for both species through NGS-CR and NGS-OM (kit fox only) approaches than had been possible through alternative monitoring strategies.

   For kit foxes, live-capture provided only relative abundance indices, as sample sizes were insufficient to estimate abundance. NGS-CR approaches successfully estimated abundance and achieved acceptable levels of precision. SECR models produced reliable estimates of density. Traditional scat surveys and scent stations produced only relative abundance indices; our NGS could produce these same relative abundance indices, but also provided information on individual ID that we used to produce quantitative estimates of abundance and survival. Additionally, NGS provided information on genetic diversity and effective population size, metrics that are not available via traditional approaches. NGS-OM for kit foxes successfully allowed inferences on habitat relationships, patterns of local colonization and extinction, and the influence of coyotes on kit foxes, all at reduced effort when compared to radio-telemetry.

   For pronghorn, we demonstrated the estimation of survival and precise abundance estimates on an annual basis, as well as the ability to evaluate genetic diversity. Current aerial monitoring methods only produce abundance estimates once every two years. For both species, parentage analyses and population genetic structure were outside the scope of the current demonstration, but such analyses could be conducted with relative ease.

2. Demonstrate that monitoring programs for kit foxes and Sonoran pronghorn based on NGS-CR provides reliable estimates of demographic parameters.

   To evaluate the success of this objective we needed to determine the precision (desired CV<10%) of the parameter estimates obtained via NGS-CR. We met the criteria of CV <10% for abundance and show that it is possible to obtain connectivity, reproduction, and genetic diversity data for both species. Therefore, we consider this objective met.

3. Demonstrate that monitoring programs for kit foxes and Sonoran pronghorn based on NGS-CR improves efficiency compared to currently implemented monitoring methods.

   For kit foxes, we obtained costs associated with alternative monitoring strategies from our collaborators at USU. Currently, kit foxes at DPG are monitored with (1) traditional scat deposition and scent post surveys, (2) live-capture and radio-telemetry, and estimates of group size, and (3) den monitoring.

   Estimated costs for annual canid monitoring based on traditional approaches was $172,291, compared to $144,652 for NGS monitoring. Canid monitoring using traditional approaches

covered 1,127 km^2 (Kluever 2015), compared to 3,663 km^2 for NGS monitoring (Lonsinger 2015). Annually, traditional approaches monitored 25–30 kit foxes and 35–40 coyotes via live-capture and radio-telemetry, whereas NGS detected 60–75 kit foxes and 201–212 coyotes. NGS monitoring conducted 2-3 times more scat deposition surveys than traditional monitoring. Using NGS-CR, we were able to increase the number of parameters estimated, spatial extent, and number of individuals monitored relative to traditional monitoring approaches, at a reduced cost. Although we conducted sampling only twice annually, compared to the year-round monitoring required for radio-telemetry, this frequency is sufficient for long-term monitoring.

For pronghorn, we obtained costs associated with current monitoring (estimating abundance biennially via aerial surveys. Survival is not estimated in this population). See section 5.6.2, and section 7.3 for pronghorn monitoring costs and estimated costs. These sections indicate NGS-CR improves the monitoring efficiency over currently implemented methods. We increased the number of parameters estimated, temporal extent of monitoring, and number of individuals monitored compared to current monitoring.

4. Demonstrate that monitoring programs for kit foxes and Sonoran pronghorn based on NGS-CR could be successfully implemented by technician-level personnel.

   We collected responses of personnel tasked with collecting field data to a Likert-type qualitative survey with statements related to (1) ability to follow field protocol, (2) ability to collect data under field conditions, (3) lack of situations encountered which prevent data collection, and (4) level of training required to collect data versus alternative approaches. We used a 5-point (1 low, 5 high) scale to score responses. We considered this objective met if the probability of obtaining a response of 4 or 5 was significantly (i.e., CIs do not overlap) greater than the probability of obtaining a response of 1, 2, or 3 for all statements. The responses from our Likert survey indicate agreement with ease of implementation of field protocol by a score $\geq$3.5, and thus our results indicate this objective was met.

5. Obtain estimates of occupancy and dynamic parameters (i.e., local colonization and extinction) via implementation of NGS-OM monitoring for kit foxes.

   We compared the cost of implementing only a NGS-OM monitoring approach to only a NGS-CR approach for the same spatial extend and time periods. We also considered the impacts on overall cost that could be ascertained by employing a combination of molecular species ID and statistical classification tree ID. We considered this objective met if (1) we were able to successfully combine NGS and occupancy modeling to generate estimates of key occupancy parameters and if (2) the cost of implementing NGS-OM was lower than implementing NGS-CR monitoring.

   Estimated costs for annual canid monitoring based on only NGS-OM was $32,502, a reduction of over $112,000 when compared to NGS-CR. Costs may be further reduced by considering the combined use of molecular species ID and statistical classification techniques, resulting in a total NGS-OM monitoring costs of $21,622. Without individual ID, NGS-OM analyses do not support estimation of parameters associated with abundance, survival, or population genetic health. NGS-OM may fail to detect important population level changes in abundance, particularly with

territorial species. NGS-OM offers a cost-effective monitoring strategy that can be used in conjunction with or in place of NGS-CR monitoring. Our criteria were met for this objective.

6. Demonstrate that personnel responsible for implementing monitoring programs for species of concern to DoD viewed NGS-CR as a preferred alternative to current approaches.

   For kit foxes, DoD managers and state biologists were evaluating monitoring approaches; both have expressed an interest in incorporating NGS as the primary monitoring strategy, or as a complimentary monitoring strategy to ongoing efforts. In Arizona, managers are implementing the NGS-CR methods for pronghorn. Using funding from DoD and USFWS, fecal samples were collected in June 2015 and 2016 to continue analyses at the University of Idaho.

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7.0 COST ASSESSMENT

Operational costs of implementing a NGS-CR-based monitoring program can be classified as either front-end costs (developing PCR tests, conducting pilot studies) or per-sample costs (ongoing costs of collecting and analyzing samples). In the cost model (Table 2), cost elements 1–3 represent one-time, front end costs, while elements 4–5 represent per-sample costs for ongoing monitoring and element 6 represents a project-level cost for quantitative analyses.

7.1 COST MODEL

Table 2. Cost Model for NGS-CR Monitoring Technology.

Cost Element	Data Tracked During the Demonstration	Estimated Costs	
		DPG	**BMGR & CPNWR**
1. Field and laboratory labor and supplies for pilot study on DNA degradation and fecal deposition	Labor and supply costs for field and laboratory	*DNA Degradation* 20 scats * 9 sampling events = 180 fecal DNA samples Field labor: 15 hours Field technician wage: $12/hour Field supplies: $250 Total field Costs: $430 Laboratory analysis at $50 per sample: $9,000 *Scat deposition/accumulation* Clear and survey of 15 5 km transects Field labor: 80 hours Field technician wage: $12/hour Field supplies: $250 Total field Costs: $1,210 Total: $10,210	*DNA Degradation* 20 scats * 8 sampling events = 160 fecal DNA samples Field labor: 5 hours Field technician wage: $12/hour Field supplies: $24 Total field Costs: $64 Laboratory analysis at $50 per sample: $8,000 *Scat deposition/accumulation* Clear 5 and survey 8 drinkers. 1 site surveyed 3x, 1 site 2x, 3 sites 1x Field labor: 6 hours Field supplies: $30 Field technician wage: $12/hour Total field Costs: $112 Total: $8,176
2. Purchasing and optimizing microsatellite primers for individual ID	Labor and supply costs for microsatellite primers and optimizing a multiplex	$3,000. This assumes 10 loci will be optimized and that loci are already developed for the species of interest.	
3. Developing species ID test	Labor and supply costs for development and optimization of species ID test	May already be developed for many species, if it needs development we estimate $3,500–$5,000.	

Table 2. Cost Model for NGS-CR Monitoring Technology (Continued).

Cost Element	Data Tracked During the Demonstration	Estimated Costs	
		DPG	**BMGR & CPNWR**
4. Field costs associated with scat sampling	Labor, travel, and supply costs required for sample collection for each demonstration site	*Based on sampling intensity at DPG for NGS-CR* • Two field technicians can survey ~15–18 km of transects daily • ~1000 hours annually/technician @ $12/hour = $24,000 • Vehicle rental and fuel for 20 weeks = $7,300 • Consumables (DETs tubes, gloves, ethanol, etc.) = $2,200 Total: $33,500	*Based on sampling intensity at BMGR for NGS-CR* • Two field technicians can collect samples at 1–3 drinkers daily • ~13.5 hours annually/ technician @ $12/hour = $324 (collection of 1000 samples) • Consumables (coin envelopes, silica, tape, etc.) = $150/1,000 samples Total: $474
5. Laboratory costs associated with fecal DNA extraction, amplification, species identification, and individual identification.	Supply use and hours required to extract DNA and complete species and individual ID	*Based on Waits lab contract rates for scat samples* • DNA extraction and species ID only: $15–$20/sample • DNA extraction and individual ID only: $30–$35/sample • DNA extraction, species ID, and individual ID: $40–$50/sample	*Based on Waits lab contract rates for scat samples* • DNA extraction and species ID only: $15–$20/sample • DNA extraction and individual ID only: $30–$35/sample • DNA extraction, species ID, and individual ID: $40–$50/sample
6. Labor associated with mark-recapture estimates	Time required to conduct mark-recapture analyses	This is difficult to estimate since it will vary based on experience and could be conducted by DoD manager or by a contractor. See below for more explanation.	

7.2 COST DRIVERS

7.2.1 Dugway Proving Ground, Utah

As demonstrated through the pilot study analyses (Lonsinger et al. 2015a), cost may be influenced by field and laboratory components. Field conditions that reduce scat detection or increase scat removal will increase field costs, while conditions that increase DNA degradation will increase both field and laboratory costs (Lonsinger et al. 2015a).

7.2.2 Barry M. Goldwater Range and Cabeza Prieta NWR

Weather and range conditions play a significant role in sample collection as drinker visitation declines in cooler, wetter conditions. This results in limited samples collected during cooler, wetter

periods. Another cost factor includes travel to more distant drinkers resulting in increased time and fuel costs. Older fecal samples have lower success rates and thus increase the cost per successful sample. Thus, optimizing sampling intervals with a pilot study can minimize costs.

7.3 COST ANALYSIS AND COMPARISON

For kit foxes, costs for implementing NGS-CR or NGS-OM via standardized transects vary by sampling frequency and intensity. Costs associated with NGS-CR monitoring was substantially lower than costs associated with more traditional strategies, while providing estimates for a greater number of parameters. NGS-OM monitoring had lower costs than NGS-CR for the same spatial extent, but yields estimates of different parameters. Field effort depends on the spatial coverage. We compare costs based on a NGS-CR sampling intensity of 150 km of multi-occasion transects and 120 km of single-occasion transects, surveyed 4x and 1x per session, respectively. We include 96 hours annually in labor estimates to set and monitor experimental scat removal plots. Total annual estimated cost to implement our sampling design is $144,652 and includes NGS-CR and NGS-OM sampling for both kit foxes and coyotes over two primary sampling periods annually. Estimates are based on collecting ~1,100 carnivore samples per session (2,200 annually), with ~25% and ~75% being kit foxes and coyote, respectively. Changes to this sampling can reduce costs, depending on management objectives. Limiting the monitoring to only kit foxes reduces costs by $49,500. Monitoring both species via NGS-CR once annually and via NGS-OM twice annually reduces costs by $56,651. Conducting only NGS-OM monitoring twice annually reduces costs by $112,150.

For pronghorn, we evaluated the efficiency of sampling methods by calculating (annual) cost per successful sample (NGS-CR) and cost per individual monitored (minimum count) in traditional aerial methods and NGS-CR methods. The number of individuals monitored was number of unique individuals identified (genotyped) in 2013 (n = 91) and 2014 (n = 110) and for traditional methods was based on minimum counts during the 2012 biennial aerial count in 2012 (n = 108) and 2014 (n = 168) conducted over ~10 days. Cost of aerial flights changes little with an increase in population size while cost of NGS-CR methods, generally increase with increasing in population size and the need to collect more samples. Using simulations, we determined what level of sampling effort would produce a CV equivalent to that from the aerial methods (CV = ~ 21%) at a true abundance equal to the 2014 aerial survey estimate (~200 individuals). We also determined at what point there was a change in cost effectiveness from one method to the other. See section 5.6.2 for a more detailed discussion of costs.

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8.0 IMPLEMENTATION ISSUES

The use of eDNA has shown considerable promise for field application in monitoring programs of rare and imperiled species. To fully understand the potential for application and limitations of this technology, three factors need to be considered: 1) production, 2) degradation, and 3) the transport or removal of eDNA. Our conceptual model (Figure 7) describes the dependency of detection on the balance between the input and output of eDNA; this model was developed in collaboration with researchers funded by the Environmental Security Technology Certification Program (ESTCP) RC-201204 and the Strategic Environmental Research and Development Program (SERDP) RC-2240.

The critical factors that determine the amount of detectable eDNA are the production of eDNA and the elimination of eDNA through degradation and removal via dilution, flow-through or capture by substrate material in the aquatic environment, and natural and human-caused physical disruptions in the terrestrial environment. The rates of production, removal, and degradation will vary by species, ecosystem, and season.

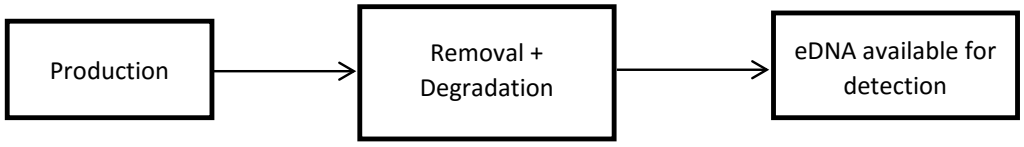

Figure 7. Conceptual Model of eDNA Production and Removal

In terrestrial environments, eDNA can be found in multiple sources (e.g., feces, hair, urine, saliva, shed skin, horns/tusks, eggshells, feathers; Taberlet et al. 1999, Waits and Paetkau 2005, Beja-Pereira et al. 2009). Here we focus on fecal samples. Deposition of eDNA with respect to fecal materials occurs at two scales: the distribution of eDNA within fecal material and the distribution of fecal material in the environment. DNA of the species depositing the feces is likely to be unevenly distributed in fecal samples and is generally at higher concentrations on the outside (Piggott and Taylor 2003, Stenglein et al 2010a). Success rates for obtaining fecal DNA vary among species, likely due to differences in rates of intestinal cell sloughing and diet (Waits 2004). At the broader scale, deposition rates and locations may vary by species due to metabolism, diet, and behavior. Within a species, there can be seasonal changes in deposition rates due to differences in diet (Smith 1964, Neff 1968, Andelt and Andelt 1984, Maudet et al. 2004), and deposition rates can vary by sex and age class (Smith 1964, Neff 1968, Todd et al. 2008, Ralls et al. 2010).

The sampling strategy of our project was designed to take advantage of species-specific deposition behavior of kit foxes and pronghorn by sampling along dirt roads and at watering holes, respectively, and included a pilot study to optimize sampling designs. We detected differences in deposition rates by season for both species and noted that deposition rates at watering holes for pronghorn were related to the degree of drought. This could lead to challenges in implementation during relatively cooler and wetter drought seasons. Also, in the pronghorn system, we are limited to estimating the size of the population using the drinkers, which varies annually due to inconsistent use as a result of climatic and range (i.e., availability of natural forage) conditions rather than true changes in population size. We do not know with certainty the proportion of the pronghorn population that uses the drinkers. However, this would be a very valuable metric and could be estimated by managers through comprehensive monitoring of the proportion of radio collared individuals using the drinkers.

In terrestrial systems, removal occurs at the scale of the discrete deposited material (i.e., scat) and is influenced by local environmental conditions, animal activity, and human activity. This rate will be higher in wetter systems because rain washes away samples (Harestad and Bunnell 1987) and wetter environments tend to have more microorganism and insect activity that breaks down the samples more rapidly (van Vliet et al. 2008, Norris and Michalski 2010). Patterns of animal and vehicular activity can affect the rate of removal by destroying scat material. We directly measured removal rates for kit fox and coyote scats and noted that removal was very high for dirt roads with increased traffic. We recommend that future monitoring avoid roads with high vehicular use and conduct sampling at times when road use is reduced.

Upon deposition, eDNA is immediately subject to biotic and abiotic forces that cause degradation and lead to additional removal even if the fecal sample remains intact. In terrestrial environments, moisture (Piggot 2004, Murphy et al. 2007, Brinkman et al. 2010), and elevated temperature (DeMay et al. 2013) increase DNA degradation rates, and ultraviolet radiation fragments eDNA (Lindahl 1993, Freidberg et al. 2003). We conducted pilot studies to specifically evaluate the rate of DNA degradation due to environmental exposure and detected differences between species and season. This pilot study was key to maximizing efficiency and effectiveness of our implementation; we believe this step should be required for all future implementations of the technology for other species and systems.

As with any sampling protocol, species ecology must be taken into account. For appropriate inference from eDNA studies, users must determine how many samples to collect and in what spatial configuration to achieve goals. Systems with lower scat deposition and/or higher removal and degradation will require increased sampling effort. Users must consider animal behavior and the spatial patterns of production and local removal and degradation when establishing the spatio-temporal sampling design. The framework provided in this document describes how these aspects interact, and we implemented our demonstration using two main sampling approaches, transect-based and targeted sampling, to increase transferability.

We see the following challenges in transferring this technology to other installations: 1) unpredictable weather and land access, 2) identification of laboratories that can do the analyses, and 3) identification of experts to conduct quantitative analyses if the necessary expertise is not present within the management team. The Waits lab is interested in future contract work with DoD to assist in implementation of this technology at other instillations.

In addition to directly benefiting the focal species and installations of this project, the resulting cost-benefit analysis, protocols, and technology transfer enables other installations to implement NGS-CR transect-based monitoring for other species of concern such as swift foxes (*V. velox*; Piñon Canyon Maneuvering Site), Island gray foxes (*Urocyon littoralis*; San Clemente and San Nicolas Island Naval Reservations), Florida panthers (*Puma concolor coryi*; Camp Blanding and Avon Park Range), and gray wolves (Camp Ripley and Fort McCoy). The standardized transect sampling approach could also be helpful for monitoring Florida black bears (*Ursus americanus floridanus*; 4 instillations). The concentrated sampling approach used for pronghorn would likely be effective for monitoring cave roosting bat species (e.g., Indiana bat, gray bat; 16 installations). While our focus was monitoring of mammals (i.e., via fecal DNA), our demonstration and development of monitoring protocols may be applied to bird species on DoD lands as well (e.g., greater sage grouse [*Centrocercus urophasianus*]). We recommend pilot projects similar to phase one of this project to evaluate the potential of the methods for other species and systems.

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